Rejection of Claims 15-17 and 59 Under 35 U.S.C. § 103(a) (Item 2 of Office Action)

Claims 15-17 and 59 have been rejected under 35 U.S.C. § 103(a), as they are said to be unpatentable over Stamler *et al.* (WO 93/09806).

Stamler *et al.* (WO 93/09806) disclose *S*-nitroso-proteins, in particular, *S*-nitroso-tPA (tPA is tissue plasminogen activator), *S*-nitroso-BSA, *S*-nitroso-cathepsin B, *S*-nitroso-lipoprotein and *S*-nitroso-immunoglobulin, and methods for producing the same, using NO or $NaNO_2$ as the reagent under acidic conditions. They also report a method which they claim results in the synthesis of *S*-nitroso-hemoglobin. However, this compound was not produced by any method reported in WO 93/09806, as attested to in the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 mailed to the Patent Office on January 6, 1999. Methods used to synthesize other *S*-nitroso-proteins, which might have been expected to nitrosate or polynitrosate hemoglobin, dissociated hemoglobin into its subunits, oxidized the heme Fe and rendered the product fragments useless for carrying oxygen. Methods described in the written description that resulted in the synthesis of nitrosated hemoglobins are substantially different from the unsuccessful acidified nitrite method described in WO 93/09806.

The Examiner states:

> Stamler et al. generally teach pharmaceutical delivery of NO by administering nitrosylated protein compounds (e.g., the addition of an NO group to an SH, oxygen, carbon or nitrogen; see page 14, lines 7-12), including nitrosylated hemoglobin, which is formed by conventional means (e.g. see Stamler page 1-5) for use in relaxing smooth muscle, inhibiting platelet aggregation, promoting vasodilation and for treating/preventing cardiovascular disorders (e.g., see page 19, lines 22-25; Stamler claims 18, 20, 36, 37, 41-42, 44, 45; etc.).

No nitrosated protein with adducts on oxygen, carbon or nitrogen was ever demonstrated in WO 93/09806 – not hemoglobin or any other protein. No data or even speculation is given as to whether proteins with adducts on an oxygen, carbon or nitrogen atom could be a donor of NO or have a physiological effect by any other mechanism. The Examiner refers to nitrosylated hemoglobin as being formed by "conventional means." Page 58, lines 4-6 of WO 93/09806 present a description of an unsuccessful attempt to produce the species of nitrosylated

hemoglobin, *S*-nitrosohemoglobin. No assay results are presented to show that *S*-nitrosohemoglobin was ever produced from this procedure. It is apparent that this method, although incompletely described, is different from the methods used to produce other *S*-nitrosoproteins described in WO 93/09806. See, for example, page 56, lines 1-2 and lines 9-11, reporting methods to produce *S*-nitroso-BSA. Therefore, it is not understood why the Examiner chooses to call the one incompletely described and unverified method of producing *S*-nitrosohemoglobin "various conventional means of making nitrosylated hemoglobin." No conventional means of producing *S*-nitrosohemoglobin had been established. The "conventional means" that had been used to produce other *S*-nitrosoproteins (the acidified nitrite method) dissociated hemoglobin into its subunits. No method of attempting to produce *S*-nitrosohemoglobin is reported to have produced any *S*-nitrosohemoglobin by any credible means of detection. Therefore, it would not have been possible, given the teachings of WO 93/09806, for one of ordinary skill in the art to use *S*-nitrosohemoglobin in a method of treating or preventing any disorder or in a method to inhibit platelet activation.

Rejection of Claims 15 and 16 Under 35 U.S.C. § 103(a) (Item 3 of Office Action)

Claims 15 and 16 have been rejected under 35 U.S.C. § 103(a) as being obvious over Stamler *et al.*, WO 93/09806 and Kaesemeyer, U.S. Patent No. 5,543,430.

The teachings of Stamler *et al.* (WO 93/09806) have been described above. Nowhere does WO 93/09806 mention nitrated proteins of any type.

Kaesemeyer (U.S. Patent No. 5,543,430) describes a method for treating cardiovascular diseases using a combination of L-arginine and a vasodilator, a compound such as nitroglycerin, for example, "which when administered to a subject is converted biologically to nitric oxide (NO) which is a pharmacologically active metabolite." See column 1, lines 21-24. *S*-nitrosothiols are mentioned as being vasodilators. See column 1, line 49. The low molecular weight nitrates isosorbide dinitrate and isosorbide 5' mononitrate are also mentioned by Kaesemeyer as sources for the production of NO. See column 6, lines 19-23, and lines 44-47. Kaesemeyer does not mention any form of hemoglobin, or any nitrosoprotein.

The Examiner states that ". . . the selection of a species of NO donating hemoglobin is a matter of choice to one of ordinary skill in the art since the Stamler reference teaches the functionally equivalent use of nitrosylated proteins, including hemoglobins, as well as individually nitrosylated hemoglobin species including thionitrosylated hemoglobin."

The Stamler WO 93/09806 reference discusses only one species of hemoglobin, *S*-nitrosohemoglobin, as possibly being a donor of nitric oxide. However, no evidence of its synthesis or of its possible biological activity is given, leaving one of ordinary skill in the art with no guidance on how *S*-nitrosohemoglobin could be made or used. WO 93/09806 does not teach or suggest nitrated hemoglobin.

One of ordinary skill in the art would find no reason in the combination of WO 93/09806 with Kaesemeyer to turn to any nitrated protein, especially any form of hemoglobin as a possible donor of nitric oxide. It was thought at the time of the invention, that if hemoglobin could be nitrated or nitrosated, the resulting nitrated protein would *block* nitric oxide activity by acting as a scavenger of nitric oxide. The heme iron of hemoglobin is known to bind NO with a very high affinity (see, for example, Greenburg, A.G. and H.W. Kim, *Art. Cells, Blood Subs., and Immob. Biotech. 23*:271-276, 1995, especially fifth paragraph on page 272; reference AX). It was also known that the administration of the combination of hemoglobin and the nitrate nitroglycerin to patients would result in methemoglobinemia (see, for example, abstract and first paragraph in Kaplan, K.J. *et al., The American Journal of Cardiology 55*:181-183, 1985; copy provided previously with the Amendment mailed to the Patent Office on May 19, 2000). Therefore, one of ordinary skill in the art would not have thought of any method of therapy using nitrated hemoglobin, as the prior art teaches adverse effects of the combination of hemoglobin and nitrate.

Rejection of Claims 15-17 and 53-59 Under 35 U.S.C. § 103(a) (Item 4 of Office Action)

Claims 15-17 and 53-59 have been rejected under 35 U.S.C. § 103(a), as they are said to be obvious over Stamler *et al.*, WO 93/09806, or alternatively, over Stamler *et al.* further in view of "the specification admission as to prior art on pages 37-39," Kaesemeyer (US Patent No.

5,543,430), Moore *et al., J. Biol. Chem. 251*:2788-2794, Sharma *et al., J. Biol. Chem. 253*:6467-72, and Wade and Castro, *Chem. Res. Tox. 3*:289-291 (1990). Claim 53 has been canceled, and redrafted as independent Claim 60. Claim 56 has been canceled, and redrafted as independent Claim 62.

The rejection of Claims 15-17 and 59 under 35 U.S.C. § 103(a) over Stamler *et al.* has been discussed above in response to item 2 of the Office Action. The rejection of Claims 53-58 under 35 U.S.C. § 103(a) over Stamler *et al.* is considered below.

Claims 54 and 55, as well as new Claim 60, are drawn to methods for treating a disorder resulting from platelet activation or adherence in an animal or human, comprising administering to the animal or human a composition comprising nitrosated or nitrated hemoglobin in a therapeutically effective amount, wherein the nitrosated hemoglobin is polynitrosated hemoglobin, methemoglobin or nitrosylhemoglobin. Claims 57 and 58, as well as new Claim 62, are drawn to methods for preventing thrombus formation in an animal or human, comprising administering to the animal or human a composition comprising nitrosated hemoglobin in a therapeutically effective amount, wherein the nitrosated hemoglobin is nitrosylhemoglobin, polynitrosated hemoglobin, or methemoglobin.

The teachings of the Stamler *et al.* (WO 93/09806) reference have been described above. WO 93/09806 discloses several *S*-nitrosated proteins, including *S*-nitroso-BSA, *S*-nitroso-tPA, *S*-nitroso-cathepsin B, *S*-nitroso-lipoprotein, and *S*-nitroso-immunoglobulin. WO 93/09806 discusses the theoretical addition of NO to O, C, or N of amino acids, but presents no evidence of this reaction occurring on any protein. WO 93/09806 gives an incomplete procedure on page 58 which is said to result in the synthesis of *S*-nitroso-hemoglobin, and suggests that *S*-nitroso-hemoglobin, could it be made, be used in methods to inhibit platelet function, like other *S*-nitrosoproteins. WO 93/09806 briefly mentions nitrosylhemoglobin (the species having NO bound to the heme Fe) on page 58, lines 19-21, but does not suggest that nitrosylhemoglobin could be a donor of nitric oxide, could be converted to a donor of NO, or have any physiological effect. WO 93/09806 does not mention any other species of hemoglobin, or suggest that other species should be made, or suggest that other species may be useful as NO donors. Therefore,

given the teachings of WO 93/09806, one of ordinary skill in the art who wanted to treat or prevent a disorder resulting from platelet activation, including thrombus formation, might turn to an *S*-nitrosoprotein other than hemoglobin, as the syntheses of other *S*-nitrosoproteins were completely described and the physiological effects of other *S*-nitrosoproteins were documented. For example, in WO 93/09806, see page 56, lines 1-12 and lines 17-24, and Figure 27, reporting the synthesis of *S*-nitroso BSA and its effects on blood vessel relaxation, indicating that nitric oxide is released from the *S*-nitroso BSA. No effects of any hemoglobin derivative are reported in WO 93/09806. Polynitrosated hemoglobin and methemoglobin are not mentioned at all. Therefore, from the teachings of WO 93/09806, one of ordinary skill in the art would not think of using nitrosylhemoglobin, polynitrosated hemoglobin or methemoglobin in any form, in any method of therapy requiring release of NO. Furthermore, hemoglobin was known at the time to activate platelets, producing thrombosis (see, for example, the fourth paragraph in Marcus, A.J. *et al., Circulation 93*:208-209, 1996; copy provided as Appendix 1).

Below is considered the rejection of Claims 15-17 and 53-59 under 35 U.S.C. § 103(a) over Stamler *et al.* and further in view of "the specification admission as to prior art on pages 37-39," Kaesemeyer (US Patent No. 5,543,430), Moore *et al., J. Biol. Chem. 251*:2788-2794, Sharma *et al., J. Biol. Chem. 253*:6467-72, and Wade and Castro, *Chem. Res. Tox. 3*:289-291 (1990). Claims 53 and 56 have been canceled and independent Claims 60 and 62 have been substituted for them.

The specification at pages 37-39 describes methods that have been used previously in nitrosating proteins other than hemoglobin.

The teachings of Kaesemeyer (US Patent No. 5,543,430) have been described above.

Sharma *et al.* (*J. Biol Chem. 253*:6467-6472, 1978) describe experiments to measure the rate of dissociation of NO from nitrosylhemoglobin. The rate constant is on the order of 10^{-4} or 10^{-5}, indicating that nitrosylhemoglobin is very stable, hence, cannot donate NO or have any physiological effect of an NO donor. Sharma *et al.* do not discuss any other species of hemoglobin.

The Moore *et al.* (*J. Biol. Chem. 251(9)*:2788-2794, 1976) reference describes experiments on nitrosylhemoglobin and nitrosylmyoglobin in which dissociation of NO from the heme Fe of these molecules is followed spectrophotometrically in the absence of oxygen. Moore *et al.* find dissociation rate constants similar to those found by Sharma *et al.* The Moore *et al.* and Sharma *et al.* papers do not report or suggest any physiological effect of nitrosylhemoglobin or any other nitrosyl-heme containing NO donor. The low dissociation constant measured for nitrosylhemoglobin -- 1,000 times lower than that of CO and 200,000 times lower than that of oxygen, by comparison (see, for example, Greenburg, A.G. and H.W. Kim, *Art. Cells, Blood Subs., and Immob. Biotech. 23*:271-276, 1995, especially fifth paragraph on page 272; reference AX) – would lead one of ordinary skill in the art to conclude that nitrosylhemoglobin cannot be a donor of NO and that nitrosylhemoglobin could have no physiological effect because of the extremely low rate of release of NO from hemoglobin. It is hemoglobin's property of scavenging NO and binding it tightly on the heme Fe (as nitrosylhemoglobin) that has been used to explain platelet activation and thrombus promotion by hemoglobin (see Marcus, A.J. *et al., Circulation 93*:208-209, 1996; copy provided as Appendix 1).

Wade and Castro (*Chem. Res. Tox. 3*:289-291, 1990) describe reactions of metmyoglobin and other oxidized heme proteins, including methemoglobin, with NO and certain organic nucleophiles, yielding nitroso products of the organic nucleophiles, but not nitroso products of hemoglobin. No reactions are reported in which the organic nucleophiles are not present. *S*-nitrosohemoglobin is not reported as a product, nor is any other *S*-nitroso derivative of a heme protein reported as a product. The synthesis of *S*-nitrosohemoglobin is not anticipated, not looked for, and not found by Wade and Castro. Wade and Castro mention nitrosylhemoglobin, but do not suggest any therapeutic function for it.

The Examiner states, ". . . the Stamler reference fails to disclose other individual species of nitrosylated hemoglobins (e.g. nitrosylhemoglobin, polynitrosated hemoglobin, and nitrosated methemoglobin)." The Examiner also states, in what seems to be a contradiction:

> Further, the Stamler *et al.* generic teaching of using NO donor nitrosylated protein compounds, including nitrosylated hemoglobins and methemoglobin, and a species of specifically nitrosylated hemoglobin (e.g. S-nitrosylated) would

> motivate one of ordinary skill in the art to utilize other nitrosylated hemoglobins which would be deemed to be functionally equivalent as NO donating compounds for use in relaxing smooth muscle and inhibiting platelet aggregation.

The Stamler WO 93/09806 reference contains no such "generic teaching of using NO donor nitrosylated protein compounds." WO 93/09806 merely speculates, without any evidence, that one species of hemoglobin, *S*-nitrosohemoglobin, would be a donor of NO if it could be made, but WO 93/09806 does not present an enabling description of how to make and use *S*-nitrosohemoglobin. WO 93/09806 does not discuss any other species of hemoglobin as possibly being a donor of NO or having any effect on platelet disorders or thrombus formation.

Furthermore, no other cited reference states that, or speculates that, nitrosated or nitrated forms of hemoglobin, in general, are donors of NO, or that they could be used in methods of therapy to treat or prevent disorders involving platelet activation or adherence.

The Examiner states:

> Applicant's argument regarding the Moore et al. and Sharma et al. reference taken alone (e.g. as not teaching NO donation of nitrosylated/nitrosated hemoglobins) is not persuasive since the teachings of these references are combined with the WO 93/09806 reference which suggests the ability of nitrosylated/nitrosated proteins (e.g. hemoglobin) to act as NO donors.

Combining the teachings of the Moore and Sharma references with the teachings of WO 93/09806, one of ordinary skill in the art would see from WO 93/09806 that there is unsupported speculation on *S*-nitrosohemoglobin, and this species of hemoglobin alone, as being a donor of NO. WO 93/09806 presents no data on any physiological effect of *S*-nitrosohemoglobin, or any other species of hemoglobin. There is no general teaching in WO 93/09806 that any other form of NO-modified hemoglobin is, or could be, a donor of NO. From studying the results of experiments shown in Moore *et al.* and Sharma *et al.*, one of ordinary skill in the art would have to conclude that nitrosylhemoglobin (that is, the species of hemoglobin with NO bound at the heme Fe), is definitely not a donor of NO, because of the very low dissociation constant of NO from nitrosylhemoglobin. The speculation about the NO-releasing properties of *S*-nitrosohemoglobin (NO bound to S of cysteine residues in β-subunit) presented in WO 93/09806 can in no way affect the conclusion of one of ordinary skill in the art who considers the data from

experiments performed on nitrosylhemoglobin, an entirely different hemoglobin species (NO bound to heme Fe). One of ordinary skill in the art, considering the teachings of WO 93/09806 regarding *S*-nitrosohemoglobin, an entirely different species of hemoglobin, would have to conclude from the experimental results in Moore *et al.* and Sharma *et al.* and other references regarding nitrosylhemoglobin, that nitrosylhemoglobin cannot act as a donor of NO, and therefore, cannot have the physiological effect of inhibiting a disorder resulting from platelet activation. The teachings of the Moore *et al.* and Sharma *et al.* references are not relevant to those of WO 93/09806 or vice versa, because the references do not describe the same species of hemoglobin.

The Examiner states:

> To the extent that applicant is arguing that only S-nitrosated hemoglobins can be used as NO donors, such an argument is inconsistent with the presently claimed invention which is not so limited nor is such an argument consistent with the Stamler WO 93 reference which suggests otherwise.

Applicants are not arguing that only S-nitrosated hemoglobins can be used as NO donors. Applicants are summarizing what is taught in the prior art. The only nitrosated species of hemoglobin that is postulated in the prior art (in WO 93/09806) as possibly being a donor of NO, should it be possible to make, is *S*-nitrosohemoglobin. There is nothing in the prior art that would suggest that nitrosated hemoglobins other than *S*-nitrosohemoglobin can be donors of NO. Nitrosylhemoglobin was known, from its high affinity for NO, to definitely not be a donor of NO.

The Examiner states further:

> Applicant argues that WO 93/09806 fails to teach a "successful synthesis of S-nitrosylated, O-nitrosylated, C-nitrosylated or N-nitrosylated hemoglobin." However, Applicant's declaration evidence directed to demonstrate nonenablement is strictly directed to a single embodiment of the Stamler reference; e.g., a single example is directed to making S-nitrosylated hemoglobin. There is no evidence of record to dispute the other Stamler reference methods provided therein regarding the syntheses of other nitrosated/nitrated hemoglobins within the scope of the presently claimed invention. In this regard, applicant is directed to the Moore et al., Sharma et al. and Wade et al. references which

> disclose methods for synthesizing nitrosated/nitrated hemoglobin species which are capable of acting as NO-donating compounds.

Applicant's declaration evidence is directed to showing that there was no synthesis of S-nitrosohemoglobin, because that species of hemoglobin was the only one which WO 93/09806 hypothesized as being produced. WO 93/09806 goes so far as to report the previously known reactions of nitrosylation and nitration of some amino acids (page 4, lines 3-13), but does not show, discuss or hypothesize the synthesis of O-, C-, or N-nitrosated hemoglobin, or any species of nitrated hemoglobin. Nowhere in the other cited references are O-, C-, or N-nitrosated hemoglobin or nitrated hemoglobin discussed in any way. The Moore *et al.* and Sharma *et al.* references describe properties only of nitrosylhemoglobin (the species which has NO bound to Fe of heme and which is *not* a donor of NO) and no other species of nitrosated/nitrated hemoglobin. The Wade and Castro reference does not describe the synthesis or properties of any nitrosated/nitrated species of hemoglobin. *S*-nitrosohemoglobin is hypothesized in WO 93/09806 as being a donor of NO, but no other species of nitrosated/nitrated hemoglobin is described as or hypothesized as being a donor of NO in WO 93/09806 or in any other cited reference. Moreover, WO 93/09806 did not predict, and could not have predicted, that SNO-oxyhemoglobin is, in fact, a vasoconstrictor. See, for example, the written description at page 90, lines 4-20, and Figure 20A.

The Examiner states:

> Turning to the Kaesemeyer reference patent, applicant argues that this reference is deficient since it fails to mention any form of hemoglobin or any nitrosoprotein. In response to applicant's arguments against the Kaesemeyer reference individually, one cannot show nonobviousness by attacking a reference individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

This is not an accurate representation of the arguments previously presented, in which the teachings of Kaesemeyer were more completely discussed, and argued in combination with the other cited references. See third paragraph on page 3 of the Amendment mailed to United States

Patent and Trademark Office on May 19, 2000, in which the combination of references is explained to be insufficient to show obviousness of the invention.

The Examiner also states:

> Applicant's argument as to what the state of the prior art was (e.g. Kaplan et al. and Greenberg references) regarding nitrosated/nitrated hemoglobin scavenger activity is not relevant to the above obviousness rejections which is directed to the ability of nitrated/nitrosated proteins, including hemoglobin, to act as an NO-donating compound as taught by the Stamler WO 93 reference.

On the contrary, this teaching in the prior art is highly relevant, as it is a teaching against the ability of nitrated/nitrosated hemoglobins to act as NO-donating compounds. If one considers that a nitrosated/nitrated hemoglobin has NO scavenger activity as well as NO donor activity, it becomes apparent that the net effect must be that the nitrosated/nitrated hemoglobin cannot be an NO donor, because the very same molecule that releases NO from one site is capturing and retaining it on another site.

The Examiner states that Applicants are focusing on only one species of hemoglobin (*S*-nitrosohemoglobin) in considering the WO 93/09806 reference, and in doing so are "impermissibly failing to consider the WO 93/09806 reference taken as a whole." Applicants focus on one species of hemoglobin (*S*-nitrosohemoglobin) in considering the WO 93/09806 reference because that species of nitrosated hemoglobin is the only one hypothesized in the reference to be a donor of NO. WO 93/09806 speaks of proteins nitrosated on sites in addition to S (oxygen, carbon and nitrogen) as a theoretical possibility (see page 1, lines 22-24 of WO 93/09806), but nowhere presents an enabling description of how to produce proteins of any kind that are nitrosated on these sites. The section pointed out by the Examiner (page 14, lines 7-12) is merely a definition of the term *nitrosylation*, and is not an enabling description of how to produce any nitrosated protein. Therefore, species of hemoglobin nitrosated on C, N, or O atoms need not be addressed, as they are not described or suggested in WO 93/09806.

The Examiner points to passages of WO 93/09806 regarding the uses of *S*-nitrosohemoglobin (page 19, lines 22-25, Claims 18, 20, 36, 37, 41, 42, 44 and 45). However, these uses are merely based on guesses about the physiological effects that *S*-nitrosohemoglobin

is hypothesized to have. WO 93/09806 does not describe the results of any experiment that would explain the physiological effect of *S*-nitrosohemoglobin. Until the priority document of Applicants, it was not known or suspected that one species of *S*-nitrosohemoglobin, SNO-oxyhemoglobin, was a *vasoconstrictor*, rather than a vasodilator as would be predicted by one of ordinary skill in the art trying to interpret the teachings of WO 93/09806.

The Examiner states, "The Stamler reference also discloses various conventional means of making nitrosylated hemoglobin to arrive at nitrosated hemoglobins within the scope of the presently claimed invention."

The Stamler WO 93/09806 reference refers (by an incomplete description in which no reagent is given) to a method that is hypothesized to produce *S*-nitrosohemoglobin. This method is incompletely described on page 58, lines 4-6: "*S*-nitrosylation of hemoglobin was accomplished by reacting 12.5 μM hemoglobin with 12.5 μM for 5 and 20 minute intervals (pH 6.9)." No assay results are presented to show that *S*-nitrosohemoglobin was ever produced from this procedure. As has been pointed out in previous Amendments, the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 (mailed to the United States Patent and Trademark Office on September 2, 1999) presents data demonstrating that if S-nitrosoacetylcysteine (SNOAc) is assumed to be the reagent, and the experiment assumed to be described in WO 93/09806 is carried out, no SNO-hemoglobin is detectable. It is apparent that this method, although incompletely described, is different from the methods used to produce other *S*-nitrosoproteins described in WO 93/09806. See, for example, page 56, lines 1-2 and lines 9-11, reporting methods to produce S-nitroso-BSA. Therefore, it is not understood why the Examiner chooses to call the one incompletely described and unsubstantiated method of producing *S*-nitrosohemoglobin "various conventional means of making nitrosylated hemoglobin." No conventional means of producing *S*-nitrosohemoglobin had been established.

The Examiner states:

> Applicant proceeds to argue that the Moore et al. and Sharma et al. references fail to suggest physiological effect or any other NO donating capability. However, it is clear from the above obviousness rejection that the Stamler reference teaching of the NO-donating ability of nitrosylated proteins, including hemoglobin, is being

> combined with the Moore and Sharma reference methods of producing nitrosylated hemoglobins within the scope of the presently claimed invention.

The Stamler WO 93/09806 reference does not contain a general teaching that every form of a nitrosated hemoglobin is a donor of NO. WO 93/09806 only refers to S-nitrosohemoglobin, should it be possible to make, as one of a number of S-nitrosoproteins that could possibly be useful in methods for causing physiological effects mediated by NO. See, for example, Claims 42-47 on pages 66-67 of WO 93/09806. Moore *et al.* and Sharma *et al.* are only two of a number of references in the prior art that describe the properties of nitrosylhemoglobin (the species having NO bound to the heme Fe) and include data showing that the affinity of the heme Fe for NO is so high as to lead one of ordinary skill in the art to conclude that nitrosylhemoglobin could not be a donor of NO. This conclusion is not contradicted by anything in WO 93/09806.

> The Examiner states further, regarding the Wade and Castro reference:
>
> As pointed out in the obviousness rejection above, the Castro et al. Res. Tox. 1990 Vol. 3, pages 289-291 reference discloses a method of transferring the nitrosyl group to sulfur (as well as oxygen, nitrogen and sulfur) of heme proteins, including hemoglobin to thus form, polynitrosated hemoglobins, including SNO-hemoglobins.

The Examiner misinterprets the Wade and Castro reference. All of the reactions described in or referred to in the Wade and Castro reference include a small organic compound which has a nucleophilic site where NO reacts to produce a *C*-NO, *N*-NO or *S*-NO adduct of the small organic compound, depending on the organic compound included in the reaction. See Scheme I on page 289 of Wade and Castro. The Wade and Castro reference does not describe products resulting from reaction at the oxygen, nitrogen or sulfur atoms of hemoglobin or any other heme protein. The only product described in the reference as resulting from a reaction of NO with heme proteins is the heme protein with a "heme-NO" adduct, that is, with NO bound to the Fe of the heme [see equation (2) on page 289 of Wade and Castro]. In the case of hemoglobin, the product postulated by Wade and Castro is nitrosylhemoglobin (in which NO is bound to the heme Fe), not SNO-hemoglobin. The other products described in the Wade and Castro reference are those resulting from NO reacting with the added reagent having a nucleophilic site – *N*-acetylcysteine, phenol, or proline. The Wade and Castro reference does not even mention a

hemoglobin nitrosated on S, O or N atoms, and therefore cannot provide an enabling description of a hemoglobin nitrosated on S, O or N atoms.

To summarize the teachings of the cited references, one of ordinary skill in the art would know from WO 93/09806 that *S*-nitrosoproteins can be vasodilators, like other *S*-nitrosothiols. One would also know from the specification and from WO 93/09806 that methods have been previously used to synthesize *S*-nitrosoproteins, but no method has been demonstrated as successful for the synthesis of *S*-nitrosohemoglobin. One would know from Kaesemeyer that administration of a combination of a vasodilator and L-arginine can be useful in treating cardiovascular disease. One would know from the Moore *et al.* and Sharma *et al.* references that nitrosylhemoglobin cannot be a donor of NO. One would know from the Wade and Castro reference that *S*-nitroso-*N*-acetylcysteine, *O*-nitroso-phenol, and *N*-nitroso-proline can be made in the presence of methemoglobin.

Combining the teachings of these references, one of ordinary skill in the art at the time of the invention might think of treating cardiovascular disease using an S-nitrosoprotein or other S-nitrosothiol that had been successfully synthesized and tested as having an effect as a vasodilator. This would exclude S-nitrosohemoglobin, which had not been synthesized and tested. Alternatively, one might choose a combination of a nitrate and L-arginine to treat cardiovascular disease. Nitrated proteins had not been described as being vasodilators or having any effect in cardiovascular disease, so nitrated proteins would not be chosen as candidates to use with L-arginine. One of ordinary skill in the art would not think of nitrosylhemoglobin as being similar to any S-nitrosoprotein or any kind of vasodilator, and would not think of administering nitrosylhemoglobin to an animal or human for any therapeutic purpose, as no therapeutic effect of nitrosylhemoglobin had been described at this time. One of ordinary skill in the art would not be able to apply any teaching of the Wade and Castro reference to the problem of a method of therapy to treat cardiovascular disease, as it describes only reactions that are not physiologically relevant.

CONCLUSION

The Examiner is respectfully requested to consider the above amendments and remarks made in response to the rejections, and to reconsider the application. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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